Patent
Attorney's Docket No. 1034005-000008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	**MAIL STOP AMENDMENT**
Henrik SEMB et al.	Group Art Unit: 1632
Application No.: 10/500,118	Examiner: T. Ton
Filed: September 14, 2004	Confirmation No.: 7902
For: METHOD FOR THE ESTABLISHMENT OF A PLURIPOTENT HUMAN BLASTOCYST-DERIVED STEM CELL LINE	

DECLARATION UNDER 35 U.S.C. § 1.132 OF DR. HENRIK SEMB

Commissioner for Patents
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Sir:

1. I, Henrik Semb, declare the following:

2. I am a citizen of Sweden, and have the following mailing address: Söderströms Väg 6, SE 23735 Bjärred, Sweden;

3. I am currently a Professor of Functional Genetics at Lund University, a position I have held since February 2004;

4. I have experience in developmental and stem cell biology, with particular expertise in human embryonic stem cell derivation, characterization, and differentiation;

5. I am a co-inventor of the invention disclosed and claimed in the above-identified United States Patent Application filed on September 14, 2004, and I am submitting this Declaration in support of that Application;

6. All the facts referred to herein are either from my own knowledge or are from my reading of documents or information provided to me by the Assignee (Cellartis AB), all of which I believe are true to the best of my information and belief;

7. The objective of my invention, which forms the subject of the Application, is to provide a method for establishing a stable line of pluripotent human embryonic stem cells, such that well characterised cells may be produced in a quantity sufficient for commercial-scale production. The method set forth in the Application is capable of generating large numbers of pluripotent human embryonic stem cells with sufficient chromosomal stability for passaging for a period of at least twenty-one months in an undifferentiated state. While the length of time for which a cell line may be passaged whilst remaining undifferentiated with a high degree of chromosomal stability is not an end in itself, the stability of the cells produced in accordance with the method of my invention allows the production of "lots" of cells, each "lot" comprising many individual units [plates, straws, *etc.*] of cells. The units of cells with each lot are similar, and it will be understood that an advantage of such a production method is that a single unit of cells may be withdrawn from a lot for the purpose of characterising all of the units within the lot. Such characterisation of the cells within each lot is highly desirable from a scientific point of view for the end-user of the cells;

8. To the best of my knowledge and belief, the Assignee, using the method of my invention as set forth in the Application, at the time the application was filed the only entity in the world that was able to produce pluripotent human embryonic stem cells in such large quantities and with a high degree of characterisation of the cells. Numerous other laboratories were working on devising methods for the production of such stable lines of undifferentiated pluripotent human stem cell lines, but at that time without success.

9. The method of my invention is also a practical one in the sense that it is easily worked. A further advantage of the pluripotent human stem cells that are produced in accordance with method of my invention is that they are free of "xeno" material, rendering them safer for administration to human patients in a potential therapeutic treatments as compared with stem cells produced by methods that involve the use of non-human materials, especially immunosurgical materials. The absence of such "xeno" material from cells produce in accordance with my invention therefore makes them particularly attractive to researchers looking at potential new therapeutic treatments in humans;

10. According to the method of my invention, colonies of inner cell mass [ICM] derived cells are manually dissected to form pieces that are then transferred to mitotically inactivated feeder cells to grow the ICM-derived cells up into colonies of blastocyst-derived stem cells. Typically, as described in the Application, such manual dissection may be performed by using a glass capillary or knife as a cutting tool, although it is envisaged that the colonies of ICM-derived cells may be manually dissected in other ways. The significance of such manual dissection is that the ICM-derived cells remain associated with one another in relatively large groups of cells (typically about one thousand cells per "piece"), and are not dissociated into individual cells or small clumps. It is thought this is important, because human embryonic stem cells, unlike murine embryonic stem cells, for example, exhibit poor clonal survival;

11. The colonies of ICM-derived cells for use in my invention are obtained by co-culturing a blastocyst-derived from a fertilized oocyte with feeder cells. It is important to use the correct starting material. It is notable that the production of colonies of ICM-derived cells for use in my invention does not necessitate removal of the trophectoderm, although the zona pellucida may be gently removed if desired. The fact that the ICM-derived cell colonies which form the starting material for the method of my invention are derived simply by co-culturing blastocysts on feeder cells without removal of the trophectoderm is advantageous, because it obviates the need for the use of xeno immunosurgical materials;

12. Thus, a first essential step of the method of my invention is mechanical dissection of the colonies of ICM-derived cells into pieces and then growing the cells within such pieces on mitotically inactivated feeder cells. The manual dissection of ICM-derived cells that have previously been obtained by co-culturing blastocysts on feeder cells does not involve the dissection of blastocysts, since the culturing of blastocysts on feeder cells leads to degradation of the other parts of the blastocysts whilst supporting growth of the ICM cells;

13. A second essential step of my invention is repeated passaging every 4 to 5 days of the blastocyst-derived stem cells that are obtained by co-culturing the ICM-derived cells on feeder cells. At each passaging step, the inner homogeneous structure of each colony of blastocyst-derived stem cells is dissected manually, again using a knife or sharp capillary as a cutting tool, to form pieces of such colonies of

blastocyst-derived stem cells that are then placed on fresh mitotically inactivated feeder cells;

14. It has surprisingly been found that the combination of the two essential steps outlined above, namely manual dissection of initial colonies of ICM-derived cells followed by repeated passaging every 4 to 5 days of colonies of blastocyst-derived stem cells obtained by co-culturing such ICM-derived cells, enables the establishment of a highly stable pluripotent human stem cell line which may be passaged for at least twenty-one months as mentioned in the Application, which also mentions the chromosomal stability of the stem cells as determined, for example, by fluorescence *in situ* hybridization ("FISH") and/or karyotyping (*see* pages 9 and 10 of the present specification). Further evidence of the chromosomal integrity of pluripotent human embryonic stem cells produced in accordance with the method of my invention is set forth in the paper by Caisander, *et al.* in Chromosome Research, 2006; 14:131-137, a copy of which has been submitted herewith;

15. Scientific understanding of the establishment of pluripotent human stem cell lines is incomplete at present, but it appears to me that both of the aforementioned steps of the method of my invention are essential, indispensable steps for producing a stable pluripotent human embryonic stem cell line with large numbers of cells. In this sense, it is impossible to identify separate technical effects or consequences associated with each respective individual step, but as a combination of steps they produce a highly successful and unexpected result which, as mentioned above, enables the Assignee to produce undifferentiated human stem cells in commercial quantities with good characterisation;

16. In this connection, experiments have shown that repeated passaging of blastocyst-derived stem cells every seven days (instead of every 4 to 5 days in accordance with the method of my invention and using the same starting material) may not lead successfully to the establishment of a pluripotent human embryonic stem cell line that is sufficiently stable to allow the production of such large quantities of cells. The reasons for this are unclear at present. In this respect, reports of the establishment of human embryonic stem cell lines published in the literature should be treated with a degree of caution, since in general, such stem cell

lines have not been followed for sufficiently long periods of time to determine their actual stability, and adverse data is not always made available;

17. Further, if a stem cell line were to be followed for a substantial period of time (*e.g.* 21 months or more) with the result that chromosomal instabilities were eventually noticed, it would still not be evident what was the cause of the introduction of such chromosomal instabilities, and it would not be certain whether a stable stem cell line could be established at all that did not possess such chromosomal instabilities, or what steps would need to be changed or modified to render such a stem cell line stable. Ultimately, the state of the art at present is such that the only way to determine whether or not a particular method for producing an human embryonic stem cell line results in the establishment of a stem cell line having sufficient chromosomal stability to allow for the production of large quantities of cells is to demonstrate empirically that such method works;

18. This is what has been achieved by the method of my invention, and, as mentioned above, to the best of my knowledge and belief there was no other methods available in the art at the time the application was filed which, when worked, would lead to the establishment of a pluripotent human stem cell line having a high degree of chromosomal stability for a period of more than twenty-one months.

19. I further declare that all statements made herein of my own knowledge are true and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2008-11-03

By: [signature]
Henrik SEMB

Curriculum vitae Henrik Semb

NAME	Henrik Semb
ADRESS	Söderströms Väg 6, 237 35 Bjärred, Sweden
TELEPHONE	+46-(0)46-294997 (home), +46-(0)46-2223159 (work), +46-(0)709-584262 (mobile)
DATE/PLACE OF BIRTH	October, 15, 1959
CITIZENSHIP	Swedish
FAMILY	Married to Kristina Forsman-Semb, 3 children: Ebba -96, Joel -92, and Fredrik -86

EDUCATION AND ACADEMIC DEGREES

1982:	Med. Kand (*Bachelor of Medicine*), completion of the preclinical part of Medical school, Umeå University
1988:	PhD (Dr. Med. Sci.), Umeå University
1989-90:	Post-Doctoral Fellow, EMBO fellowship, Department of Biochemistry and Biophysics, UCSF, San Francisco, California, USA
1997:	"Docent" in Medical Molecular Biology, Umeå University

APPOINTMENTS

1982-88:	Predoctoral student, Department of Physiological and Medical Chemistry, Umeå University
1991-97:	Assistent Professor (forskarassistent), Department of Microbiology, Umeå University
1997-98:	Associate Professor (lektor), Department of Microbiology, Umeå University
1998-2003:	Associate Professor (lektor) in developmental biology, Institute of Medical Biochemistry, Göteborg University
2003-2004	Professor in developmental biology, Institute of Medical Biochemistry, Göteborg
2004-present	Professor in functional genetics, Stem Cell Center, Lund University,
2006-08	Assistant director of the Stem Cell Center, Lund University
2008-present	Director of the Stem Cell Center, Lund University

COMISSIONS OF TRUST

Member of Medical Advisory Committee of the Swedish Research Council (2002-present)

Medical Advisory Committee of the Juvenile Diabetes Research Foundation (2003-present)

"Ad hoc" reviewer: MRC (UK), and Inserm (France)

Elected Swedish human embryonic stem (ES) cell expert in an international committee (International Stem Cell Forum) with a global joint initiative to enhance progress in basic human embryonic stem cell research (2004-present).

Member of the Editorial Board of *Stem Cells* (2008-present), *Regenerative Medicine* (2005-present
"Ad hoc" reviewer: Nature, The EMBO Journal, Diabetes, Journal of Cell Biology, Development, Journal of Cell Science, Mechanism of Development, Developmental Dynamics, Stem Cells

NETWORKS IN ACADEMIA AND INDUSTRY (ALL IN BETA CELL/DIABETES FIELD)

EU funding

6th FP (integrated project, work package leader of the ES cell project (incl NovoNordisk+Cellartis AB): " *BETACELLTHERAPY*" (2005-04-01) Coordinator: Danny Pipeleers (Daniel.Pipeleers@vub.ac.be) (2005-2010)

NIH funding

Subproject leader in a U19 project (PI: Palle Serup) within Beta Cell Biology Consortium (BCBC), NIH (2005-2009)

JDRF funding

PI for program project grant (2004-2007)

VR (Swedish Research Council) funding

Co-PI for Linné grant, Lund University Diabetes Center (2006-2016)

ENTREPENEURAL ACHIEVEMENTS

Co-founder of Cellartis AB, member of board of directors, scientific advisor. Cellartis is a Gothenborg-based company that commercializes human ES cell applications. Several patents filed.

Publication list

ORIGINAL ARTICLES

1. **Semb, H.,** and Olivecrona, T. Nutritional regulation of lipoprotein lipase in guinea pig tissues. *Biochimica et Biophysica Acta* 876, 249-255, 1986.
2. **Semb, H.,** and Olivecrona, T. Lipoprotein lipase in guinea pig tissues: molecular size and rates of synthesis. *Biochimica et Biophysica Acta* 878, 330-337, 1986.
3. **Semb, H.,** Peterson, J., Tavernier, J., and Olivecrona, T. Multiple effects of tumor necrosis factor on lipoprotein lipase *in vivo*. *Journal of Biological Chemistry* 262, 8390-8394, 1987.
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5. **Semb, H.,** and Olivecrona, T. Mechanisms for turnover of lipoprotein lipase in guinea pig adipocytes. *Biochimica et Biophysica Acta* 921, 104-115, 1987.
6. Enerbäck, S., **Semb, H.,** Tavernier, J., Bjursell, G., and Olivecrona, T. Tissue-specific regulation of guinea pig lipoprotein lipase; effects of nutritional state and of tumor necrosis factor on mRNA levels in adipose tissue, heart and liver. *Gene.* 64, 97-106, 1988.
7. **Semb, H.,** and Olivecrona, T. The relation between glycosylation and activity of guinea pig lipoprotein lipase. *Journal of Biological Chemistry* 264, 4195-4200, 1989.
8. **Semb, H.,** and Olivecrona, T. Two different mechanisms are involved in nutritional regulation of lipoprotein lipase in guinea pig adipose tissue. *Biochemical Journal* 262, 505-511, 1989.
9. Fager, G., **Semb, H.,** Enerbäck, S., Olivecrona, T., Jonasson, L., Bengtsson-Olivecrona, G., Camejo, G., Bjursell, G. and Bondjers, G. Hyperlipoproteinemia typeI in a patient with active lipoprotein lipase in adipose tissue and indications of defective transport of the enzyme. *Journal of Lipid Research* 31, 1187-1197, 1990.
10. Sjödin, A., Dahl, U., and **Semb, H.** Mouse R-cadherin: expression during the organogenesis of the pancreas and gastro-intestinal tract. *Experimental Cell Research* 221, 413-425, 1995.
11. Dahl, U., Sjödin, A., and **Semb, H.** 1996. Cadherins regulate aggregation of pancreatic β-cells *in vivo*. *Development* 122, 2895-2902.
12. Eng, H., Herrenknecht, K., **Semb, H.,** Starzinski-Powitz, A., Ringertz, N., and Gullberg, D. Effects of divalent cations on M-cadherin expression and distribution during primary rat myogenesis in vitro. *Differentiation* 61, 169-176, 1997.
13. Rosenberg, P, Esni, F., Sjödin, A., Larue, L., Carlsson, L., Gullberg, D., Takeichi, M., Kemler, R., **Semb, H.** R-cadherin is involved in striated muscle formation. *Developmental Biology* 187, 55-70, 1997.
14. Perl, A. -K., Wilgenbus, P., Dahl, U., **Semb, H.,** and Christofori, G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 392, 190-193, 1998.

15. Esni, F., Perl, A. -K., Täljedal, I.-B., Cremer, H., Christofori, G, and **Semb, H.** Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. *Journal of Cell Biology* 144, 325-337, 1999.
16. Perl, A. -K., Dahl, U., Wilgenbus, P., Cremer, H., **Semb, H.**, and Christofori, G. Neural cell adhesion molecule (N-CAM) modulates the metastatic dissemination of pancreatic β tumor cells. *Nature of Medicine* 5, 286-291, 1999.
17. Esni, F., Johansson, B. R., Radice, G. L., and **Semb, H**. Dorsal pancreas agenesis in N-cadherin-deficient mice. *Developmental Biology* 238, 202-212, 2001.
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19. Fagman, H., Grande, M., Edsbagge, J., **Semb, H.**, and Nilsson, M. Expression of classical cadherins in thyroid development: maintenance of an epithelial phenotype throughout organogenesis. *Endocrinology* 144, 3618-24, 2003.
20. Edsbagge, J., Zhum S., Xiao, M.Y., Wigstrom, H., Mohammed, A.H., and **Semb, H.** Expression of dominant negative cadherin in the adult mouse brain modifies rearing behavior. *Molecular and Cellular Neuroscience* 25, 524-35, 2004.
21. Stahlberg, A., Hakansson, J., Xian, X., **Semb, H.**, and Kubista, M. Properties of the reverse transcription reaction in mRNA quantification. *Clin. Chem*. 50, 509-15. 2004.
22. Heins, N., Englund, M.C.O., Sjöblom, C., Dahl, U., Tonning, A., Bergh, C., Lindahl, A., Hanson, C., and **Semb, H.** Derivation, characterization and differentiation of human embryonic stem cells. *Stem Cells* 22, 367-76, 2004.
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25. Edsbagge, J., Johansson, J., Esni, F., Luo, Y., Radice, G., and **Semb, H**. Vascular function and sphingosine-1-phosphate regulate development of the dorsal pancreatic mesenchyme. *Development*, 132, 1085-1092, 2005.
26. Håkansson, J., Xian, X., He, L., Ståhlberg, A., Nelander, S., Samuelsson, T., Kubista, M., and **Semb, H**. N-CAM-deficient β cell tumourigenesis results in diminished ECM molecule expression and tumour cell-matrix adhesion. *Tumor Biology* 26, 103-12, 2005.
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29. Brolén, G.K.C., Heins, N., Edsbagge, J., and **Semb, H**. Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta cell-like cells. *Diabetes* 54, 2867-2874, 2005.

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36. Adewumi et al. Characteristics of Human Embryonic Stem Cell Lines: Results from the International Stem Cell Initiative. *Nat Biotech.* **25**, 803-16, 2007.
37. Ståhlberg, A., Bengtsson, M., and **Semb, H.** Quantitative transcription factor analysis of undifferentiated single human embryonic stem cells. *Genome Res.* In review.
38. Kesavan, G., Greiner, T., Wolfhagen, F., and **Semb, H.** Cell polarity coordinates tubulogenesis and cell differentiation during pancreas development. In preparation.
39. Greiner, T., Kesavan, G., and **Semb, H.** Members of the Rho GTPases regulate pancreatic islet cell migration. Submitted.
40. Wolfhagen, F., Johansson, J., and **Semb, H.** Sphingosine-1-phosphate signaling is required for epithelial-mesenchymal interactions during pancreas development. In preparation.
41. Ameri, J. Johannesson, M., Ståhlberg, A., Norrman, K., and **Semb, H.** FGF-signaling patterns hESC-derived gut endoderm into pancreatic endoderm in a concentration-dependent manner. In preparation.
42. Fischer, Y., Xian, X., and **Semb, H.** Generation of a GFP-Nanog reporter hESC line by homologous recombination in hESCs. In preparation.

Invited Reviews

1. Olivecrona, T., Price, S.R., Pekala, P.H., Scow, R.O., Chernick, S.S., **Semb, H.,** Vilaro, S., and Bengtsson-Olivecrona, G. 1987. Regulation of lipoprotein lipase activity. Its role in lipid lowering therapies. *Drugs affecting lipid metabolism IV*, Springer Verlag, Heidelberg, Ed. by Paoletti, R., Kritcheusky, D., and Holmes, W.L. pp 88-93.

2. **Semb, H.** and Christofori, G. The tumor suppressor function of E-cadherin. *American Journal of Human Genetics* **63**, 1588-1593, 1998.
3. Christofori, G., and **Semb, H.** The functional role of the cell adhesion molecule E-cadherin as a tumor suppressor gene. *Trends in Biochemical Sciences* **24**, 73-76, 1999.
4. Christofori, G., and **Semb, H.** The functional role of the cell adhesion molecule E-cadherin as a tumor suppressor gene. *Trends in Biochemical Sciences* **24**, 73-76, 1999.
5. **Semb, H.** Human embryonic stem cells: origin, properties and applications. *APMIS*, **113**, 743-50, 2005.
6. **Semb, H.** Definitive endoderm from embryonic stem cells. *Regenerative Med.* **1** (4), 2006.
7. Gerhardt, H. and **Semb, H.** Pericytes: Gatekeepers in tumour cell metastasis? *J. Mol. Med.* **86**, 135-144, 2008.
8. **Semb, H.** Definitive endoderm: a key step in coaxing human embryonic stem cells into transplantable beta-cells. *Biochem Soc Trans.* **36**(Pt 3), 272-5, 2008.

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Semb, H. Cadherins in development. Handbook of Experimental Pharmacology. Cell adhesion, Springer-Verlag, Ed. By Behrens, J., and Nelson, W.J. Vol 165, 53-68, 2004.

Thesis

Semb, H. LIPOPROTEIN LIPASE. Synthesis, turnover and regulation. Medical Dissertation. 1988-04-13.

Popular science articles

Semb, H. 2002. Tänkbar framtida behandling av diabetes. Källa 54: Livets urcell. Fem forskares syn på stamcellsforskning.Vetenskapsrådet. Ed. Leander, G. pp41-51 (Swedish).